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Frequency dependence of muscarinic facilitation of transmitter release in urinary bladder strips from neurally intact or chronic spinal cord transected rats

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1 Electrical stimulation evoked release of ^{3}H-noradrenaline (NA) and ^{14}C-acetylcholine (ACh), as well as neurally evoked contractions were measured at various (1–40 Hz, 100 shocks) stimulation frequencies in bladder strips from neurally intact (NI) and spinal cord transected (SCT) rats.

2 The frequency response curves for ACh and NA release were shifted to the left in SCT bladder strips as compared to NI bladder strips.

3 Atropine (1 μM) depressed ACh release in NI bladder strips at high frequency stimulation (10 and 40 Hz) but not at low frequency stimulation (2–5 Hz). However, in SCT bladders, atropine depressed ACh release both at low and high frequencies of stimulation, indicating that muscarinic facilitation occurs at lower frequencies.

4 Atropine depressed the release of NA in NI bladders at only 40 Hz stimulation, but depressed release at all frequencies in SCT bladders.

5 The amplitude of neurally evoked contractions of bladder strips from NI rats was enhanced as the frequency of stimulation was increased from 1 to 40 Hz (80 shocks). The frequency response curve was shifted to the left in SCT bladders. Atropine blocked the neurally evoked contractions in SCT bladder strips to a greater extent than the contractions in NI strips indicating a cholinergic dominance in the SCT bladders.

6 Maximal contractile force of SCT bladder strips evoked by neural stimulation at 20 Hz 10 shocks and 80 shocks was significantly lower than that of NI bladder strips, whereas the release of ACh was significantly higher in SCT than NI bladders indicating a postjunctional defect in the SCT preparations.

7 It is suggested that presynaptic muscarinic facilitatory mechanisms are upregulated in the cholinergic and adrenergic nerve terminals in SCT bladders leading to a larger relative contractile response at lower frequencies of stimulation (2–5 Hz). Thus the hyperreflexic bladder occurring after spinal cord injury may be due in part to an enhancement of transmitter release at bladder postganglionic nerve terminals.

Keywords: Urinary bladder; spinal cord transection; ACh; NA; release

Introduction

Spinal cord transection abolishes voluntary control of voiding as well as coordinated urinary bladder/urethral sphincter activity producing bladder sphincter dyssynergia and functional obstruction (de Groat *et al*., 1997). Chronic outlet obstruction increases the bladder mass (Kruse *et al*., 1995) and the levels of neurotrophic factors in the bladder (Steers *et al*., 1991). These changes are accompanied by increased excitability of bladder afferent neurons which is due to plasticity of sodium and potassium channels (Yoshimura & de Groat, 1993; 1997).

Spinal cord injury also seems to affect the efferent pathways to the bladder. Bladder strips obtained from spinal cord injured rats exhibited more prominent responses during electrical field stimulation (Yokota & Yamaguchi, 1996) which might be due to increased release of ACh. In urinary bladder strips from normal rats the release of acetylcholine (ACh) and noradrenaline (NA) from postganglionic nerve terminals is modulated by muscarinic receptors, either inhibitory M_2 or M_4 receptors (D'Agostino *et al*., 1986; 1997; Somogyi & de Groat, 1990; 1992) or facilitatory M_1 receptors (Somogyi & de Groat, 1992, Somogyi *et al*., 1994; 1996). Thus it is possible that the cholinergic dominance in neurally evoked bladder contractions after spinal cord transection may be due to increased facilitated release of ACh from bladder postganglionic terminals. To test this we examined the muscarinic modulation of ACh and NA release in bladder strips obtained from neurally intact (NI) and spinal cord transected (SCT) rats. The frequency dependence of presynaptic modulation was evaluated by using a range of frequencies from 2–40 Hz. In addition, the neurally evoked contractions were also measured at different frequencies of stimulation in both types of bladders to examine how the putative change in ACh release modifies the electrically evoked contractile responses. Some of these data were published in an abstract (Zernova *et al*., 1996).

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Methods

Surgery

Female Sprague Dawley rats (250–300 g) were anaesthetized with halothane and the spinal cord was completely transected at T8–9 level. Ampicillin (150 mg/ kg^{-1}) was administered i.m. for 3 days postoperatively. Bladders were emptied twice a day by manual compression (Kruse *et al*., 1995) until the emergence of automatic bladder function (10–14 days) The rats were used for experiments 3–4 weeks after the operation.

Preparation

The urinary bladder was removed from neurally intact (NI) or spinal cord transected (SCT) rats following decapitation with a guillotine (Harvard, Apparatus Inc. Dover, MA, U.S.A.) using the protocol approved by the Institutional Animal Care and Use Committee of University of Pittsburgh. The bladder was removed from the abdomen, and four circular strips of 10–15 mg were prepared from the middle part of the bladder body by cutting the bladder transversely. Special care was taken not include the bladder base or the dome of the bladder. The circular strips were cut open and were used either for release studies or mounted in a double jacketed organ bath and used for the contractile experiments.

Experiments on NA and ACh release

A double labeled isotopic technique was used to measure simultaneous release of ^{3}H-NA and ^{14}C-ACh from the same bladder strip. Strips were incubated consecutively in Krebs solution containing 10 μCi ml^{-1} levo (7-^{3}H)-NA (specific activity: 17Ci mmol^{-1}) for 30 min followed by a brief washout period and then incubated for another 30 min with 0.5 μCi ml^{-1} (methyl-^{14}C)-choline (specific activity: 50 mCi mmol^{-1}) at 36°C. The Krebs solution during the incubation contained 50 μM ascorbic acid and 10 μM EDTA-Na_2. After the incubation the strips were suspended in a bath and superfused at a rate of 0.3 mL min^{-1} with oxygenated Krebs solution (mmol L^{-1}): NaCl 113, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.5 and constantly bubbled with the mixture of 95% O_2 and 5% CO_2 After a 60 min washing period, 3 min effluents were collected with a fraction collector for 33 min.

Stimulation and drug administration

Electrical field stimuli were delivered with a Grass 88 stimulator (Astro-Med, West Warwick, RI, U.S.A.) through platinum plate electrodes positioned on the top and the bottom of the perfusion bath and separated by 25 mm. A different stimulation paradigm was applied in the release and contractile experiments. In the release experiments the electrical field stimulation consisted of 100 shocks (100 V and 0.25 msec pulse duration) at 2, 5, 10 and 40 Hz in the 66th min of the superfusion. Drugs were added 20 min before the stimulation. In the contractile experiments platinum wire electrodes were inserted from the bottom and the top of the tissue bath and positioned such that the tips were 4 cm apart. Two successive frequency response curves were constructed with trains of 10 and 80 shocks respectively applied at 1, 2, 10, 20 and 40 Hz. In bladders from NI rats stimulation either with low frequencies (1–5 Hz) or stimulation at high frequencies (10–40 Hz) with small number of shocks (10) was considered to be a non-facilitatory stimulation; whereas, stimulation at high frequencies (10–40 Hz) with 80–100 shocks was regarded as facilitatory stimulation based on previous studies of Somogyi *et al*. (1994) which measured ACh release

Calculation of the experimental results

The radioactivity in the effluent was measured using Scintisafe biodegradable scintillation reagent (Fisher Scientific, Fair Lawn, NJ, U.S.A.) with a Beckman Scintillation Beta Spectrometer (Fullerton, CA, U.S.A.). The measured counts were corrected to absolute activity both for ^{14}C and ^{3}H using the computer program supplied with the scintillation counter. The acid soluble radioactive content of tissue slices for both isotopes was determined after the experiments by placing the tissue in 1 mL of 10% perchloric acid for 16 h prior to counting. The released amounts of ^{3}H and ^{14}C are expressed as the fraction of the tissue ^{3}H and ^{14}C content, respectively (fractional release). The evoked release of NA and ACh was computed by calculating the area below the increased efflux curve of ^{3}H and ^{14}C, respectively as described previously (Somogyi *et al*., 1996).

Contractile experiments

Bladder strips weighing 20–30 mg were mounted in a double jacketed organ bath at 36°C in Krebs solution. The initial tension was set to 10 mN and isometric contractions were measured with strain-gauge transducers and recorded with a computerized data acquisition program. (Windaq, DATAQ Instruments Inc, Akron, OH, U.S.A.). The amplitude and the area of the stimulation evoked contractions were computed by the WindaxEx program (DATAQ, Akron, OH, U.S.A.).

Drugs

Atropine sulfate, ascorbic acid, ethylene-diamine-tetraacetic acid disodium salt: dihydrate, and all constituents of the Krebs solution were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Perchloric acid was obtained from Aldrich Chemical Co (Milwaukee, WI, U.S.A.). ^{14}C-choline and ^{3}H-noradrenaline were purchased from DuPont, NEN (Boston, MA, U.S.A.).

Statistical analysis

The data were analysed by paired *t*-test, and unpaired *t*-test by using the Prism Statistical Program (Graphpad, San Diego, CA, U.S.A.). A level of $P<0.05$ was considered statistically significant. The data are expressed as mean and $\pm$ s.e. mean of the '*n*' number of experiments.

Results

Frequency dependence of facilitation of ACh and NA release

Bladder strips were stimulated at 2, 5, 10 and 40 Hz with 100 shocks and the release of labeled ACh and NA was measured. As shown in Figure 1 there was a positive correlation between the frequency of stimulation and the release of ACh and NA both in NI and SCT bladders. However, there was a significant difference between the frequency response curves in SCT and NI bladder strips. In NI bladders ACh release gradually increased as the frequency of stimulation was increased from 2–40 Hz and the release reached its highest value at 40 Hz. In SCT rats the frequency response curve was shifted to the left and there was a sharp increase of ACh release at 5 Hz and the release reached its maximum at 10 Hz. At 5 and 10 Hz the release was significantly higher in SCT than in NI rats ($P<0.05$). However, at a higher frequency of stimulation (40 Hz) the release was not increased further and the maximum ACh release was similar in both NI and SCT bladders. As shown in Figure 1B, NA release in NI rat bladder strips was also frequency dependent. In the bladders of SCT rats the frequency response curve was shifted to the left and the

fractional release of NA was higher at every frequency in comparison to release in NI rats.

Atropine (1 μM), a nonspecific muscarinic blocker applied 20 min before the stimulation reduced the facilitated release of ACh to the level of the non-facilitated release at every frequency of stimulation both in SCT and NI bladders (Figure 1). In NI bladders, atropine significantly blocked ACh release at 10 and 40 Hz, whereas, in SCT bladders the release of ACh was also significantly reduced both at 2 and 5 Hz. NA release in NI bladders was not reduced by atropine in the range of 2–10 Hz stimulation, but was significantly inhibited at 40 Hz. In contrast in SCT bladders NA release was inhibited by atropine at every frequency of stimulation.

The uptake of choline in SCT bladders was slightly lower (by 16%) than in NI bladders ($34{,}093 \pm 1838$ Bq g^{-1} and $40{,}600 \pm 2313$ Bq g^{-1}, $n=12$, respectively $P<0.05$), the uptake of NA was substantially lower (by 50.2%) in SCT bladders than in NI bladders ($180{,}700 \pm 10{,}112$ and $363{,}700 \pm 8685$, respectively $n=12$, $P<0.05$).

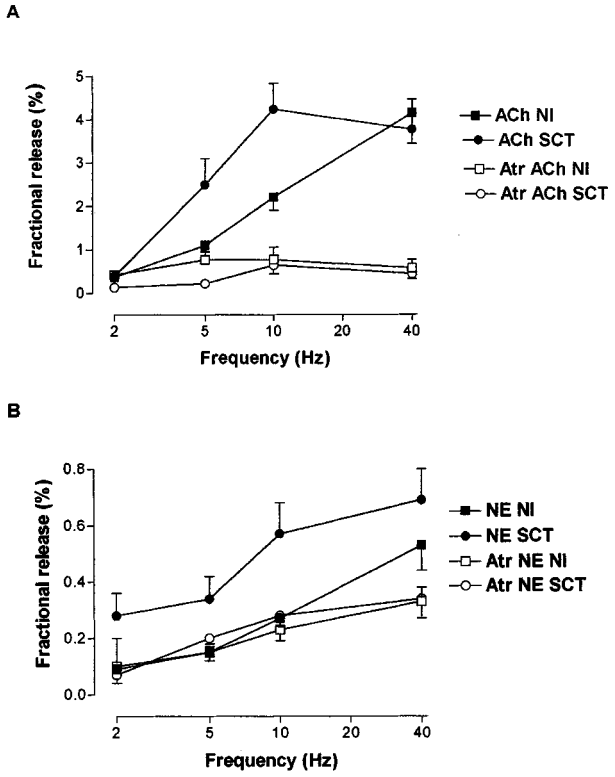


Figure 1 Frequency dependence of ACh (A) and NA (B) release in bladder strips from neurally intact (NI) and spinal cord transected (SCT) rat. The release of ACh and NA was evoked by 100 shocks delivered at different frequencies. The frequency of stimulation was plotted against the fractional release of ACh or NA. The data are expressed as means $\pm$ s.e.mean. NI non-treated (■) $n=11$; SCT non-treated (●) $n=11$; NI atropine treated (□) $n=5$; SCT atropine treated (○) $n=5$. In (A) the release values in NI and SCT bladders are significantly different from each other at 5 and 10 Hz. $P<0.05$). Atropine significantly blocked ACh release in NI bladder strips at 10 Hz and 40 Hz ($P<0.05$), however, in SCT bladders atropine significantly blocked ACh release at all frequencies of stimulation ($P<0.05$). Note: although it cannot be seen in the figure there is a significant difference ($P<0.05$) between ACh release in atropine treated and non-treated bladder strips at 2 Hz stimulation. In (B) NA release was significantly higher at 5, 10 and 40 Hz in SCT than in NI bladders. Atropine significantly blocked NA release at 40 Hz ($P<0.05$) in NI bladders, however it was inhibitory at all frequencies in SCT bladders.

Frequency dependence of the electrically evoked contractions

Bladder strips from SCT and NI rats were stimulated at a frequency of 1, 2, 5, 10, 20 and 40 Hz with the same number of shocks (10 and 80 shocks). The amplitudes of the neurally evoked contractions are expressed as a multiple (fold increase) of the amplitude of contractions at 1 Hz, 10 shocks. By normalizing the contraction amplitudes in this way the variation between the experiments was reduced and more homogeneous results were obtained. When stimulation was produced by 10 shocks at different frequencies in bladder strips from NI rats the amplitude of the contractions increased gradually up to 10 Hz, and then declined slightly at 20 and 40 Hz (Figure 2A). In bladder strips from SCT rats the amplitude reached a maximum at 5 Hz and there was a slight but not significant decrease in the amplitude at higher frequencies of stimulation. The normalized amplitudes of contractions in the SCT rats were lower, than that in the NI rats. However, during facilitatory stimulation (80 shocks at higher frequencies), the frequency dependent increase in the contractions was higher in SCT rats than in NI rats reaching a maximum at 40 Hz in NI rats and at 10 Hz in SCT rats. As shown in Figure 2B the frequency response curve was shifted to the left in SCT rats which correlates with the data obtained in the experiments with ACh release.

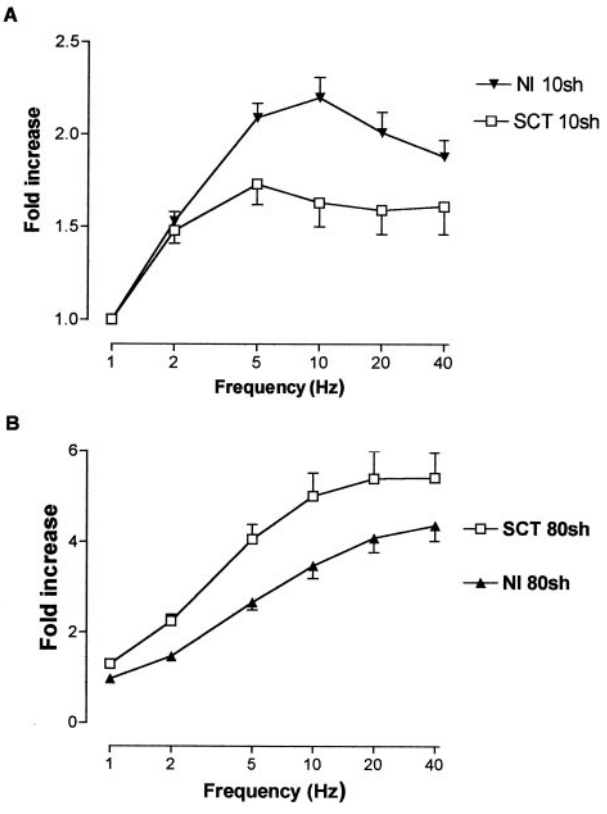


Figure 2 Contractile response of bladder strips from neurally intact (NI) and spinal cord transected (SCT) rats evoked by electrical stimulation delivered at various frequencies with 10 shocks (A) and 80 shocks (B). The contractile responses of the individual experiments were expressed as the ratio (fold increase) of the measured contractile response above the amplitude at 1 Hz stimulation. The frequency of stimulation was plotted against the fold increase values. The data in the Figure are expressed as means $\pm$ s.e.mean of the normalized data. NI bladder $n=5$; SCT bladder $n=5$. Note: a logarithmic scale was used for the abscissa.

Another way to analyse the data is to compare the relative amplitude of the contractions induced by non-facilitatory (5 Hz, 10 shocks) and facilitatory stimulation (40 Hz, 80 shocks) when the duration of stimulation was fixed (2 s). When 40 Hz/5 Hz ratios were analysed using the contraction amplitude NI strips clearly exhibited significantly smaller ratio ($P<0.05$, 2.12 ± 0.18) than SCT strips (3.89 ± 0.25) (Figure 3). However, this frequency difference was even more robust when the areas of the contractile curves were compared at 5 and 40 Hz using 2 s train duration. As shown in Figure 3 the 40 Hz/5 Hz ratio calculated from the contraction area (3.89 ± 0.25 for the NI and 7.25 ± 0.75 for the SCT bladder strips; $P<0.05$; Student t-test) was significantly higher than that calculated from the amplitude.

However, the contractile force (absolute amplitudes) of neurally evoked contractions of the NI bladder strips evoked by the non-facilitatory stimulation paradigm (20 Hz 10 shocks) or by the facilitatory stimulation paradigm (20 Hz 80 shocks) were significantly higher ($P<0.05$) than those of SCT bladder strips (Table 1) suggesting that the smooth muscle contractions are less efficient despite the higher release of transmitters in the SCT bladders.

Effect of the muscarinic blocker, atropine on the electrically evoked contractions

Atropine (1 μM) reduced the normalized amplitudes of contractile responses evoked by 80 shocks in NI and SCT rats (Figure 4). However, the inhibitory effect of atropine was higher in SCT rats than in NI rats. When the effect of atropine was evaluated on the basis of the 40 Hz/5 Hz ratio calculated from the area under the contractile curves, atropine produced a much higher inhibitory effect in SCT bladders than in NI bladders (56 and 26%, respectively; Figure 5).

Discussion

The urinary bladder in the rat undergoes a profound morphological and functional plasticity after spinal cord injury. After the initial phase of spinal shock, a spinal reflex

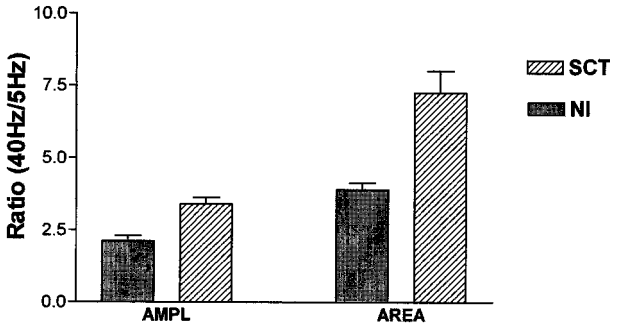
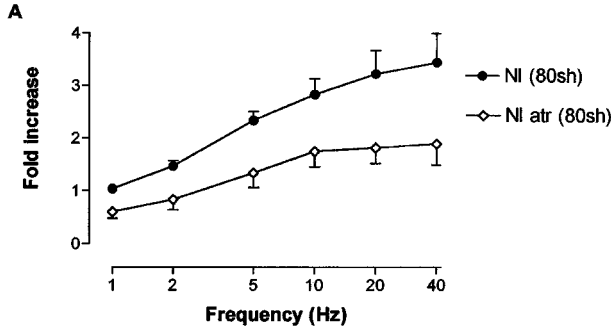


Figure 3 The 40 Hz/5 Hz ratio calculated from the amplitude and the area of the contractile curves from NI and SCT rats bladders. Stimulation was applied for 2 s at 5 Hz and 40 Hz. The amplitude and area were computed using the Statistic feature of the WindaqEx playback program. The ratios indicate the relative effect of the frequency-dependent facilitation on the amplitude and area of the contractile curves. Note that facilitation had a greater effect on the area than on the amplitude of contractions.

Table 1 Effect of the number of shocks on the amplitude of neurally evoked contractions in SCT and NI bladder strips

	Amplitude of contractions	
	20 Hz/10 shocks (mN mg^{-1})	*20 Hz/80 shocks* (mN mg^{-1})
NI bladders	0.60 ± 0.4 (12)	2.08 ± 0.27 (10)
SCT bladders	0.20 ± 0.03 (12)*	0.43 ± 0.09 (12)*

Contractions of bladder strips were induced by two stimulation paradigm 20 Hz/10 shocks or 20 Hz/80 shocks in preparations obtained from neurally intact (NI) and spinal cord transected (SCT) animals. Asterisks denote significant difference ($P<0.05$; Student t-test) between contraction amplitudes measured in NI and SCT bladder strips.



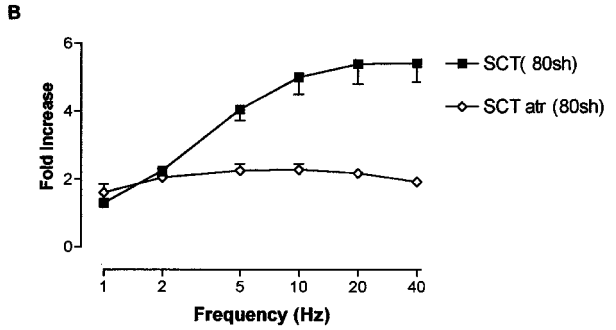


Figure 4 The blocking effect of atropine (1 μM) on the normalized contractions of the bladder strips taken from NI (A) and SCT (B) rats. The data are means$\pm$s.e.mean of five experiments.

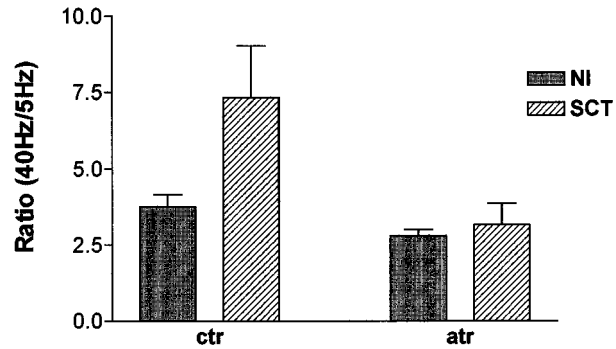


Figure 5 Depressant effect of atropine on the area of the neurally-evoked contractile responses of the bladder strips from NI and SCT rats. The 40 Hz/5 Hz ratio was computed from the area values from each contractile curve. The strips were stimulated for 2 s with 5 Hz and 40 Hz frequencies. The data are mean$\pm$s.e.mean ($n=11$ for control; $n=5$ for atropine treated strips).

pathway emerges that mediates automatic micturition and hyperreflexia (de Groat, 1995). In addition, coordination between the external urethral sphincter and the bladder is lost resulting in functional bladder outlet obstruction and an increase in bladder mass (Kruse *et al*., 1995) which is accompanied by changes in the properties of the bladder afferent pathway (Yoshimura & de Groat, 1997). In this paper we have shown that the alterations in bladder function after spinal cord transection are also accompanied by changes in presynaptic modulation of transmitter release at the adrenergic and cholinergic nerve terminals leading to changes in neurally evoked bladder contractions.

As shown in earlier studies ACh and NA release was facilitated in NI bladder strips *via* M_1 muscarinic receptors at high frequency of stimulation (10 Hz) when 100 shocks were delivered continuously, whereas, the same number of shocks given as an intermittent stimulation (10 shock trains delivered every 5 s) at the same frequency produced a much lower 'non-facilitated' release of ACh and NA (Somogyi *et al*., 1994; 1996). The present experiments extended this analysis to lower frequencies of stimulation (2–5 Hz). In strips from NI rats ACh and NA release was not facilitated at 2 Hz 100 shocks, but some facilitation occurred at 5 Hz and more prominently at 10 and 40 Hz. Thus there is a positive correlation between the degree of facilitation and the stimulation frequency. However, when the frequency dependence of ACh release was analysed in the SCT bladder strips it was noted that the release was facilitated at 2 Hz and reached a maximal facilitation at 10 Hz. Thus the frequency response curve was shifted to the left in SCT rats.

The frequency response curve for NA release showed a similar shift to the left after spinal cord injury, but in SCT bladder strips in comparison to NI bladder strips the release was markedly higher at every frequency including 2 Hz and 40 Hz. Thus in SCT bladders the facilitation of NA release seems to be shifted to the left to a higher degree than that of ACh raising the possibility that the muscarinic receptors in the adrenergic terminals are more sensitive than those in the cholinergic terminals. However, another factor also should be taken into consideration, namely that the uptake of NA was 50% lower in SCT bladders than in NI bladders which results in higher fractional release values for the same NA release, suggesting that the NA release is overestimated in the SCT bladders relative to the NI bladders. On the other hand this also means that relatively more NA is released from lower tissue NA stores which in turn supports the notion that the release of NA is higher from the SCT bladders. The function of adrenergic system in SCT bladders needs further investigation.

Since facilitation of ACh and NA release is inhibited by atropine or low concentrations of the specific M_1 receptor blocker, pirenzepine, it is believed to be mediated by M_1 muscarinic receptors (Somogyi *et al*., 1994; 1996). In this study atropine was used to determine the degree of presynaptic M_1 facilitation of transmitter release. The release of ACh at 2 and 5 Hz stimulation in NI bladders was unchanged in the presence of atropine indicating that M_1 facilitation was not important under these conditions. However, ACh release was reduced by atropine at 10 and 40 Hz indicating that the release was facilitated at these frequencies. Because atropine suppressed ACh release in SCT bladders even at 2 Hz the M_1 facilitation must occur at a lower frequency range in SCT bladders strips. A similar change occurred with NA release. NA release was only suppressed by atropine at 40 Hz in NI bladder strips, whereas, in SCT preparations atropine reduced the release at all frequencies.

The influence of presynaptic facilitation on neurally evoked bladder contractions was also evaluated in the present study. In this set of experiments the contraction amplitudes were expressed as a multiple of the contractions elicited by 1 Hz stimulation, which cannot induce facilitation of ACh release in the bladder. During facilitatory stimulation with 80 shocks the normalized contractile response was higher in SCT than in NI bladders strips. On the other hand, during non-facilitatory stimulation (10 shocks) at different frequencies the normalized amplitude of the contractions in NI bladders was higher than that of SCT bladders. It is probable that the contractile responses evoked by 10 shocks reflect primarily a purinergic component and that the cholinergic component is negligible; whereas, during stimulation with 80 shocks the cholinergic component is more prominent. This difference is likely to be exaggerated when the area of the contractile curve instead of contraction amplitude is used to measure the contractile response since the cholinergic contraction is more prolonged than the purinergic contraction (MacKenzie & Burnstock, 1984; Maggi *et al*., 1985; Brading & Mostwin, 1989).

In the present experiments contractions were elicited by 2 s stimulation at 5 Hz (10 shocks) which is a non-facilitatory stimulus and 2 s at 40 Hz (80 shocks) which is a facilitatory stimulus (Somogyi *et al*., 1994). Under these conditions it is assumed that the 40 Hz/5 Hz area ratio will indicate the degree of facilitation. When the 40 Hz/5 Hz ratio was calculated for both the contraction amplitude and the area (Figure 3) the area ratio was higher for both the NI strips and SCT strips. This indicates that the area measurement is a more sensitive way to express the facilitation when the contractile response of the bladder strips is measured. Atropine suppressed the area of the contractile curves to a greater extent in the SCT than in the NI bladders (Figure 5), a finding that is in a good agreement with a recent report of Yokota & Yamaguchi (1996) indicating that in bladder strips taken from spinalized rabbits atropine had a greater blocking effect on the electrically evoked contractions than in strips from NI rabbits.

However, the mechanism for the left shift of the frequency response curves in SCT bladder strips still remains to be explained. One possible explanation is that the presynaptic facilitatory muscarinic receptors are upregulated in SCT bladders and thus the facilitation occurs in a lower frequency range (2–5 Hz) where normally there is no or very slight facilitation of ACh release. As a result there is a more intense muscarinic facilitation of ACh release at 2–5 Hz stimulation and a more prominent effect of atropine to inhibit ACh release in SCT bladder than in NI bladder strips. On the other hand it is possible that the signal transduction mechanism mediating presynaptic facilitation is upregulated in SCT bladders resulting a more intense muscarinic facilitation of transmitter release at lower frequencies. Finally, down regulation of presynaptic inhibitory M_2 receptors is also a possible alternative in SCT bladders. The down regulation of presynaptic M_2 muscarinic receptors under pathological circumstances has been previously described in the lung (Fryer & Jacoby, 1991). Further investigation is required into whether the M_2 receptor down regulation occurs in SCT bladders.

It might be expected that higher release of ACh in the SCT bladders would translate into stronger bladder contractions. However, our results do not support this conclusion. In spite of the higher facilitated release of ACh during electrical stimulation, the contractile force produced by neural stimulation was significantly lower in SCT than in NI bladder strips. This suggests that the postjunctional mechanisms in SCT bladders are altered in such a way that higher release of neurotransmitters does not translate into proportionally larger

contractions. The postjunctional change may be due to lower expression of the postsynaptic M3 muscarinic receptors and/or the impaired function of the smooth muscle contractile mechanism.

In summary, the present experiments provided a possible explanation for the cholinergic dominance in the bladders of spinal cord injured rats. This dominance may be due to increased operation of presynaptic muscarinic facilitatory mechanisms. The more intense facilitation of ACh release at the cholinergic nerve terminals in SCT bladders and the impaired function of the smooth muscle may explain the uninhibited, inefficient contractions of neurogenic bladders.

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